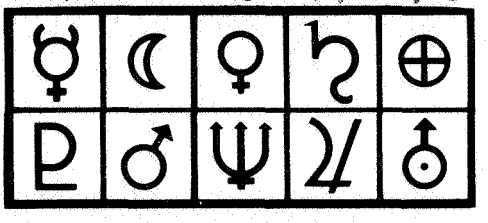


PLANETARY QUARANTINE

QR 11

December 1968

SANDIA LABORATORIES QUARTERLY REPORT - PLANETARY QUARANTINE PROGRAM

Planetary Quarantine Department 1740

SANDIA LABORATORIES

OPERATED FOR THE U. S. ATOMIC ENERGY COMMISSION BY SANDIA CORPORATION | ALBUQUERQUE, NEW MEXICO; LIVERMORE, CALIFORNIA

Sandia Laboratories Quarterly Report - Planetary Quarantine Program

Eleventh Quarterly Report of Progress

for

Period Ending December 31,1968

Planetary Quarantine Department

Sandia Laboratories, Albuquerque, New Mexico

December 1968

Project Nos. 0064010
0064020

This work was conducted under NASA Contract Numbers R-09-019-040 and H-13245A.

TABLE OF CONTENTS

I. Contamination Control Study (NASA Contract No. H-13245A)

A. Description. This project involves preparation of the NASA CONTAMINATION CONTROL HANDBOOK. The handbook assembles in one document reliable technical information and data and other information pertinent to the field but not readily available from any one source. It further provides practical information applicable to specific contamination control situations.

Included in this study activity is the additional requirement to revise and expand Section 8 and 9 of the handbook as originally submitted to the Marshall Space Flight Center (MSFC) last quarter. This in turn necessitated revisions to the Index page and the Glossary. The additional work described above was authorized by Amendment No. 4 to the NASA contract, which extended the contract completion date to June 30, 1969.

B. Progress. The levels of accomplishment during the quarter are illustrated by the following activities:

1. Extensive research was conducted in the areas of: (1) Clean packaging materials, including their characteristics, uses, and methods of application, and (2) activities involved in

maintaining product cleanliness through handling, storage, transport and field operations. During this phase of the project, discussions were held with representatives of the following NASA agencies and companies:

Manned Spacecraft Center, Houston, Texas

Kennedy Space Center, Cape Kennedy, Florida

TRW, Inc., Redondo Beach, California

North American Rockwell Corporation,
Cape Kennedy, Florida, and Downey and Canoga Park,
California

Grumman Aircraft Co., Cape Kennedy, Florida

McDonnell-Douglas Corp., Cape Kennedy, Florida

The Richmond Corporation, Redlands, California

2. The information developed was reviewed and verified when necessary, and assembled into the standard handbook format. With the addition of the new material which provides comprehensive coverage of these subjects, Section 8 was expanded from 10 to 43 pages and Section 9 from 3 to 7 pages. In order to more appropriately describe the revised material, the title of Section 8 was changed from Packaging to Clean Packaging and the title of Section 9 was changed from Handling and Storage to Maintaining Product Cleanliness.

3. A revised Index page for the entire handbook was prepared.

4. The revision of the Glossary, Section 11, was completed by adding a number of new terms.
5. All of the revised material mentioned above has been typed on masters and again reviewed for technical and editorial correctness. Preliminary printed copies will be available for NASA about January 6, 1969.

As a matter of information on the progress of the first printing of the handbook by MSFC, the following information is provided:

1. NASA Tech Brief 68-10392, describing the handbook and its availability, was distributed in late November 1968.
2. Two hundred copies were prepared on the first printing by MSFC.
3. Requests for copies resulting from the Tech Brief had exceeded the number available by about December 20.
4. A second printing, which will incorporate all of the revised material, is anticipated in late January 1969.

II. Bioburden Experimentation and Modeling

A. Description. Models for the estimation of spacecraft bioburdens have been developed for use in "dirty" areas associated with manned lunar missions (see QR 10). Three areas of activity remain. First, these models should be compared with data taken in highly controlled experimental situations which have, at the same time, sufficient flexibility to allow model parameters to be altered and measured and are reasonably accurate representations of spacecraft contamination situations. Secondly, a predictive bioburden model must be developed for use in relatively "dirty" environments, and this must be consistent with data obtained in similar highly controlled experimental situations. Finally, the models need to be refined and expanded for use in "clean" environments for ultimate use in planetary programs. An experimental program guiding parameter selection and yielding data for verification of these latter models is needed.

B. Progress. In order that physical data be available for model verification in each of the cases mentioned above, a recirculating downflow unit with the following properties was desired. The unit should be designed so that:

1. Uniform airflow in a downward direction is provided to simulate airflow conditions in laminar downflow clean rooms.
2. Test particles can be added to the airstream so that they are uniformly distributed throughout the test area, and constant

particle densities are maintained for the duration of a given test which may run to several hours.

3. Test particle sizes and concentrations can be varied to meet test requirement conditions.
4. Test particles "tagged" with tracer organisms can be used as the test particles described above.
5. The test area be sufficiently large not to bias experiments due to boundry airflow disturbances.

Such a unit has been designed and is now operational. This unit has an 8' x 8' x 8' space available for test surface exposure. It also has a capability of variable velocity airflow up to 150 feet/minute. The acoustic particle disseminator described in Sandia Report QR-9, June 1968, was installed and tested in the return and plenum of the recirculating unit.

Aluminum oxide particles, ten microns in size, were used to test performance of the downflow unit. A modified Royco particle counter was used to test particle concentrations for 5 microns and larger particles. Data for a typical test run of 20 minutes was 3000 particles per cubic foot with less than 10% particle count variation. The unit and dust feeder have been operated for periods of several hours at concentration levels as high as 11,000 particles per cubic foot.

The aluminum oxide particles will be tagged with Bacillus subtilis spores as the test organism for these tests. The "tagging" technique utilizing mechanical mixing as described in Sandia Report QR-9, June 1968, is being used.

Currently, the behavior of the unit is being studied over large ranges of values for airflow velocity, particle size, particle density and percentage of tagged particles. It should be possible to obtain data for use in the verification of the models reported in QR-10 in the near future.

A predictive bioburden model, as the phrase is used here, should be distinguished from an estimative model. The distinction is that models which estimate bioburdens on surfaces do so at some point in time based upon surface samples taken at that point in time. Predictive models can predict bioburdens in two ways. The burden may be predicted at a given point in time based upon a past estimate and subsequent environmental samples, or the burden at some future point in time may be predicted based upon a past or current bioburden estimate and current and past environmental data taken in areas where the surface will be located before the future time at which the prediction is required. A preliminary version of a model to predict in both these ways, for use in "dirty" environments, has been completed.

III. SLA, IU and SIVB Contamination Calculations

A. Description. The SLA, IU and SIVB portions of any Apollo mission are unlikely to come into contact with the lunar surface. On the other hand, should they do so, these portions may present a contamination situation rather different from previous unmanned hardware impacting the moon. This difference arises in part from the difference in size, but more importantly from the fact that impact location is not likely to be known. Under these circumstances, it was desirable

to have a better understanding of the possible effects that terrestrial organisms on the SLA, IU and SIVB might have on subsequent lunar exploration.

B. Progress. Using the previous analysis done by M.S. Tierney ("The Chances of Retrieval of Viable Microorganisms Deposited on the Moon by Unmanned Lunar Probes", Sandia Laboratories Monograph, SC-M-68-539) estimates were made of the dispersal of fragments of the SLA, SIVB and IU upon impact with the lunar surface. It was judged that only the burial of contaminated fragments posed a potential long term contamination hazard (see reference). Thus, the zones in which SLA, SIVB or IU fragments may reside are, long term, the areas of concern to planetary quarantine.

If s represents the distance from impact of a SLA, SIVB or IU, then the probability of a fragment lying at or beyond s, denoted $p(\geq s)$, from the impact site of any of these may be calculated as a function of the following parameters:

1. M, the mass of the spacecraft portion,
2. VI, the impact velocity of the spacecraft portion,
3. FKE, the fraction of kinetic energy imparted to the fragments
4. MO, the mass of the smallest fragment, and
5. NF, the number of fragments.

Impact velocity was assumed to be the escape velocity ($\sim 2.6 \times 10^5$ cm/sec), M was varied between 5×10^6 and 1×10^8 grams. Based upon the above

report, FKE was set at 1×10^{-4}. The values of MO were varied about 0.2 grams based upon the results of high velocity rocket impact in basalt and NF varied between 1×10^{6} and 1×10^{8}. Typical results obtained are shown in the following tables. Here $p(< s) = 1 - p(\geq s)$, and M/NF is used as a parameter since the model dependence is upon this ratio rather than its individual components in the ranges discussed above.

Table 1: M/NF = 10 (gm), MO = 0.2 (gm)

s (km)	p(< s)
0.01	0.0240092938
0.10	0.1981088311
1.00	0.7205374680
2.00	0.8439226322
5.00	0.9396992231
10.00	0.9753034403
20.00	0.9921656748
50.00	0.9992247444
100.00	0.9999607904
200.0	0.9999997586
300.00	0.9999999975
400.00	0.9999999999+

Table 2: M/NF = 1 (gm), MO = 0.2 (gm)

s (km)	p(< s)
0.01	0.0282350311
0.10	0.2321586643
1.00	0.8188939567
2.00	0.9344357668
5.00	0.9930904084
10.00	0.996737156
20.00	0.9999985982
30.00	0.9999999919
40.00	0.9999999999

From the tables it may be seen that if one has no more than 10^8 fragments, then for $1 \leq M/NF \leq 10$ (gm) the expected number of fragments lying beyond 300 km from the point of impact is less than one. Thus, certainly outside of a circle of a conservative radius of 300 km about the impact point of a SLA, SIVB or IU long term contamination would appear to be no problem. The comparable figure for smaller unmanned probes given in the above referenced document is 60 km based on experimental data suggesting an M/NF ratio of at most a few grams for comparable size rockets. Since similar data for rockets the size of a SIVB are not available it is possible that 60 km is a reasonable value for it and the SLA as well.

The likelihood of the impact of a SLA, SIVB or IU appears to be unknown ("reliable" estimates varying from about 10^{-3} to 0.38) and should one of these items impact the moon, the location of the impact site will also probably not be recorded.

Under these conditions it is reasonable to ask what effect their impact might have upon biological exploration. As a first step in assessing this, Table 1 may be extended to yield approximate organism densities about a lunar impact point. Based upon an estimate of 10^8 organisms on a SLA, SIVB or IU at impact (which is high, based on PHS data taken at Cape Kennedy), Table 3 gives an average expected number E(n) of organisms deposited in annular regions (with interior and exterior radii listed under Δs) about the impact point. This

assumes essentially a uniform distribution of organisms on the spacecraft portion. The area, A, of each annular region is listed and an average density, ρ_A, for the region calculated.

Table 3: Typical Average Organism Density

Δs(km)	E(n)	A(m^2)	ρ_A(org/cm^2)
0.00-0.01	2.40×10^{6}	3.14×10^{2}	7.64×10^{-1}
0.01-0.10	1.74×10^{7}	3.11×10^{4}	5.61×10^{-2}
0.10-1.00	5.23×10^{7}	3.11×10^{6}	1.68×10^{-3}
1.00-2.00	1.23×10^{7}	9.42×10^{6}	1.30×10^{-4}
2.00-5.00	9.58×10^{6}	6.60×10^{7}	1.45×10^{-5}
5.00-10.00	3.56×10^{6}	2.36×10^{8}	1.51×10^{-6}
10.00-20.00	1.69×10^{6}	9.42×10^{8}	1.78×10^{-7}
20.00-50.00	7.06×10^{5}	6.60×10^{9}	1.07×10^{-8}
50.00-100.00	7.36×10^{4}	2.36×10^{10}	3.12×10^{-10}
100.00-200.00	3.90×10^{3}	9.42×10^{10}	4.13×10^{-12}
200.00-300.00	23.9	1.57×10^{11}	1.52×10^{-14}
300.00-400.00	0.24	2.20×10^{11}	1.09×10^{-16}

Beyond about 12 km, the average density of organisms is less than $10^{-6}/cm^2$. If one assumes a uniform fragment distribution at this distance, the probability of retrieval of one of these organisms per square centimeter of surface sampled is also considerably less than 10^{-6}. This figure is often used as a criterion for successful lunar biological exploration. To complete the examination of the likelihood of retrieving organisms deposited by a SLA, SIVB or IU, one needs only consider the likelihood of retrieving them within 12 km of the impact point of any of these.

The actual assessment of the probability that a future lunar sample taking mission will land within 12 km of a previously impacted SLA involves many unknowns (among them such things as the dependence of the SLA's location on the trajectory of the mission from which it came and the subsequent similarity between the trajectory of this mission and the sampling mission of concern). Looking at only a second Apollo mission following the first, much of this difficulty can be avoided by restricting the impact points of previous mission elements and the second mission touch-down to the Apollo Landing Zone (ALZ). This becomes an approximation to the "worst case". Assume that the previous mission elements are independent of one another (which is an incorrect but conservative assumption in this context) and that their impact points are randomly placed within the ALZ. Then the likelihood of encountering any of the above annular regions within 12 km of SLA, SIVB or IU impact points become essentially

$$P_R = \frac{\text{Area of annular region, R}}{\text{Area of the ALZ}}$$

Table 4 indicates P_R for the annular regions above. Assuming a uniform density within these regions $P_R \rho_A$ gives the approximate likelihood of retrieving an organism in a cm^2 sample in each region, given that the previous mission elements SLA, SIVB, IU <u>did</u> impact the moon.

Table 4: Likelihood of Retrieval

R	P_R	$P_R \rho_A$
0.00-0.01	10^{-10}	7.6×10^{-11}
0.01-0.10	10^{-8}	5.6×10^{-10}
0.10-10.00	10^{-6}	1.7×10^{-9}
1.00-2.00	3×10^{-6}	3.9×10^{-10}
2.00-5.00	2×10^{-5}	2.9×10^{-10}
5.00-10.00	7.5×10^{-5}	1.1×10^{-10}
10.00-20.00	3×10^{-4}	5.3×10^{-11}

The probability of retrieving an organism in a cm^2 sample within a circle of radius 20 km of the impact point of a SLA, SIVB or IU is approximately the sum of the items in column 3 which is approximately 3×10^{-9}. Thus, the likelihood of retrieving any organism from the SLA, SIVB or IU per square centimeter of sample on the next Apollo mission never exceeds 10^{-6}, independent of the probability of impact of these mission items.

IV. Sterilization Modeling and Laboratory Support Work

A. Description. This investigation is directed toward sterilization modeling and support experimentation to the extent sufficient for confidence in generating the bioengineering parameters necessary for attaining planetary quarantine sterilization objectives.

B. Progress. Experimentation done this quarter suggests that another environmental parameter can be added to the growing list of factors influencing dry heat sterilization. Our initial thinking was that samples removed from the oven should be brought to room temperature as quickly

as feasible. The rationale was that the sterilizing reactions should be stopped at the moment samples are removed. Intuitively, it seemed that more spores would be sterilized during a longer cooling period. However, a comment by Saul Kit in Kinetics of Thermal Degradation of Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA), Vol. 3, No. 4, Biochemical and Biophysical Research Communications; namely, that both physicochemical and biological evidence exist that renaturation takes place with bacterial DNA preparations provided that cooling is gradual; suggested that our intuition might well be 180° out of phase. Therefore, an experiment was initiated to study the effect of different cooling rates. The samples were exposed at 135°C for the same periods of time, one set was allowed to cool to room temperature in approximately two minutes while the other set was brought to room temperature in 15 seconds. The results, shown in Graph 1, confirm that our intuition was indeed out of phase.

These results coupled with the comment by Kit suggest an experiment, to be underway shortly, for the purpose of investigating the effect of prolonged cool down on microbial survival. This is potentially very important to planetary quarantine in that an extended cooling period is indicated for spacecraft sterilization.

Since a rational model is based on what is occurring physically and we deem such a model necessary for attaining planetary quarantine objectives, an experiment directed toward the goal of better understanding the nature of microbial dry heat sterilization was performed

this quarter. It was observed that the activation energies required for the inactivation of typical enzymes were from two to three times higher than those usually seen for the inactivation of DNA and RNA. It was noted in QR 9 that the activation energies found by our model roughly agree with in vitro results for DNA-RNA. This indicated that if our model is approximately correct, from the rational viewpoint, then after sterilization some enzymatic activity should remain in the sterilized spores. To experimentally check this hypothesis derived as a consequence of the model analysis, it was noted that enzymes are necessary in order that a spore become a vegetative cell. The process of germination can be indirectly observed by continuously measuring the optical density of a population of spores as it becomes a population of vegetative cells. A population of spores is optically much denser than an equal number of vegetative cells. As the cells later multiply, the optical density again increases.

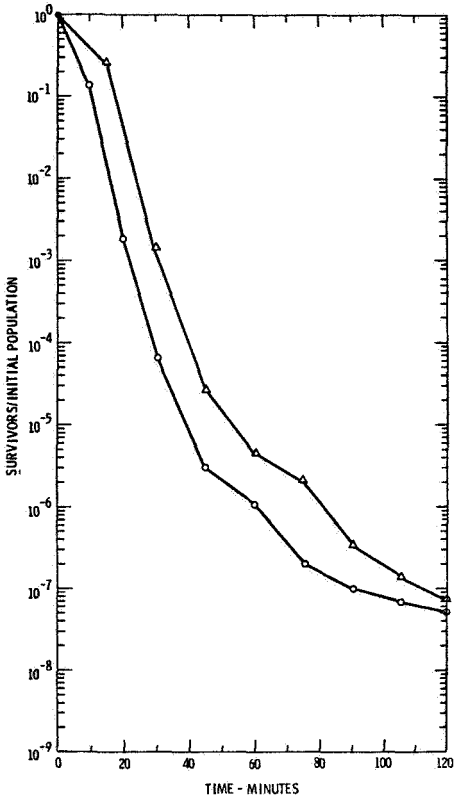
The optical densities of 3×10^8 spores heated for 60 minutes at $135^\circ C$ and an unheated sample of 3×10^8 spores are compared in Graph 2. Note that the OD of the heated sample roughly follows that of the unheated sample (after an initial lag) until germination begins in the unheated case. For the reasons outlined above, this may be interpreted as an indication that enzyme deactivation is not a major factor in dry heat sterilization.

Computer computations were performed to study the effect of prolonged heating at reduced temperature levels and to investigate the consequences

of slow heat up and cool down. The parameters used in these computations were those of QR 9 in which the base line data; survival data at 125°C, 135°C and 145°C; were from tests in which the samples were cooled to room temperature in 15 sec. Thus, the previously mentioned consequence of rapid cooling is in effect and the results of these computations would not apply directly to spacecraft sterilization. The results of these computations are given in Graphs 3 and 4.

Based upon the model predictions given in Graph 3 there is the suggestion that heating at a reduced level for an extended period of time, on the order of five to ten days depending on the temperature, could be effective in attaining sterilization objectives.

In examining the theoretical effects of heat up and cool down times, three temperature profiles were used. In each case, there was a linear heat up and cool down period between 60°C and 125°C: The differences arising in the amounts of time taken to heat up to and cool down from 125°C and the time spent at 125°C. Graph 4 shows the results of these calculations. In all cases, no appreciable decrease in population is observed when the temperature is below the range of 100°C to 105°C. In all cases, the number of survivors is proportional to the total heat ("integrated lethality") to which the organisms were exposed.

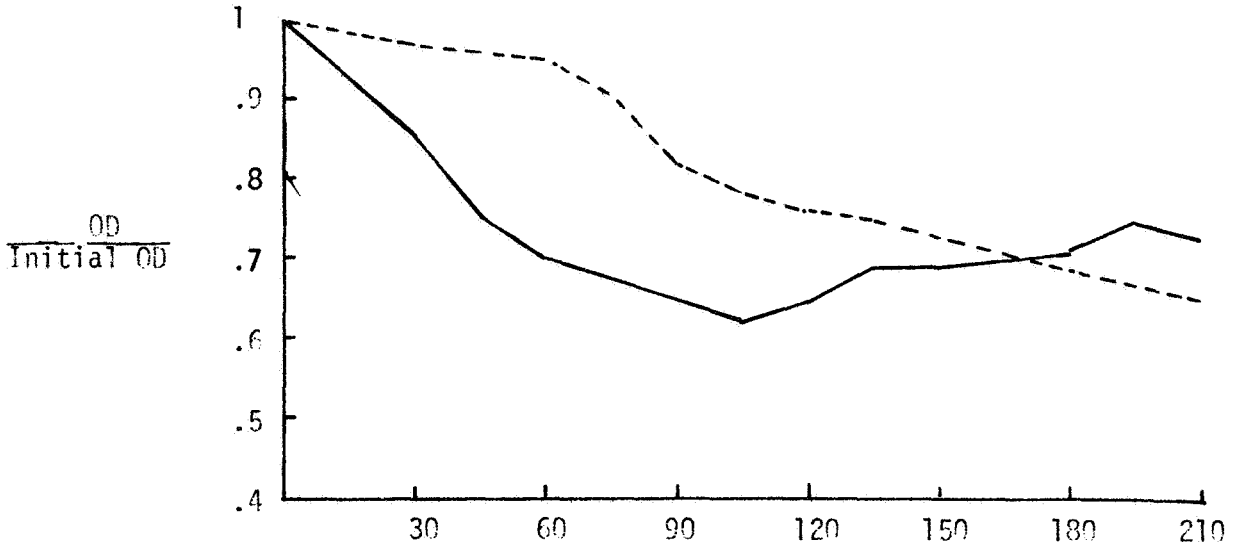


Slow cool down of sample vs. fast cool down

0-0 indicate curve resulting from rapid cooling of the sample

Δ-Δ indicate slow cooling sample upon removal from oven.

Graph 1

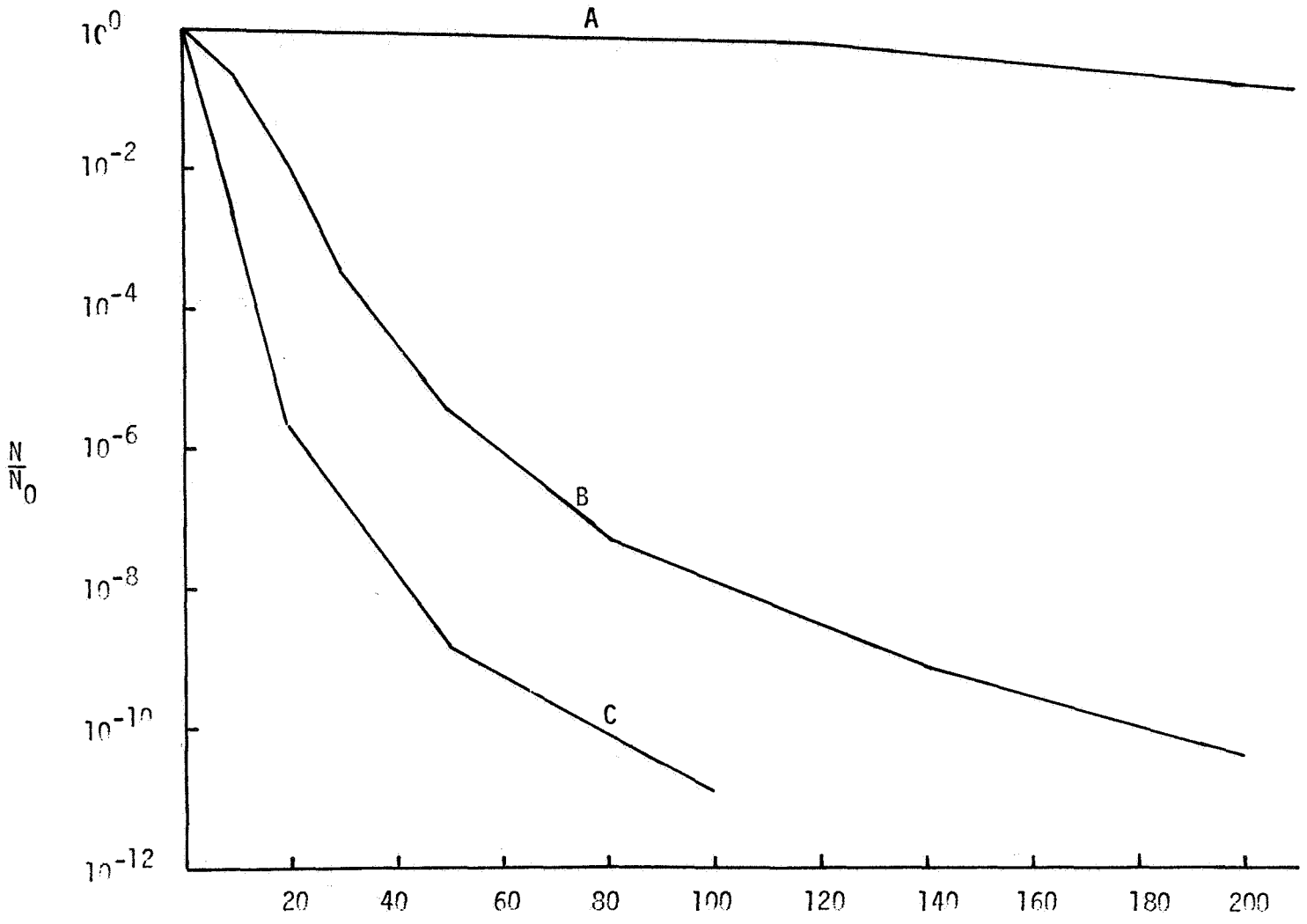


Optical density of heated and unheated samples.

------ Heated sample

——— Unheated sample

Graph 2

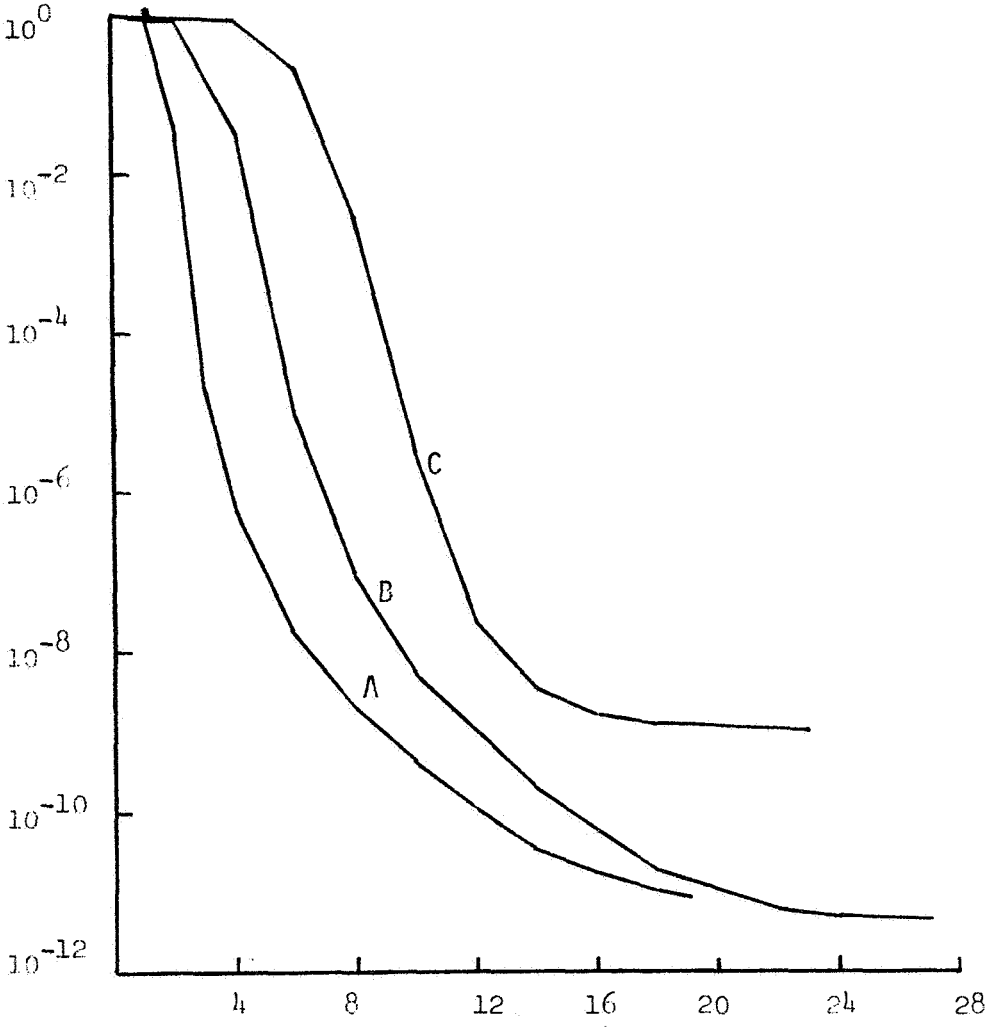


Effect of prolonged exposure at low temperature

Time of exposure in hrs

Curve	Temp
A	$60^{o}C$
B	$90^{o}C$
C	$100^{o}C$

Graph 3



Effect of variable temperature

All times in hours

Curve	Heat up time	Time at 125°C	Cool down time
A	2	15	2
B	6	15	6
C	10	3	10

Graph 4

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